The endocrine axes of fish and amphibians share common key events identified using the concept of Adverse Outcome Pathways (AOP)

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1. Introduction

In order to be defined as endocrine disrupting chemical, a substance has to meet a number of criteria, including the induction of adverse effects, specific endocrine mode-of-action and plausible link between these. Especially the latter criterion might not always be unequivocally determined, particularly as the endocrine system consists of diverse endocrine axes, e.g. the hypothalamus-pituitary-gonadal (HPG-) axis, the hypothalamus-pituitary-thyroidal (HPT-) axis, and the hypothalamus-pituitary-adrenal/interrenal (HPA/I-) axis. These axes closely interact with each other, and manipulation of one is not without effects on the other.
For mechanistic understanding of these interactions, the concept of Adverse Outcome Pathway (AOP) might be used. Therefore, in this study common key events (KE) and key event relationships (KER) shared by two or more endocrine axes were identified by focusing on fish and amphibians, with side views on mammals, as most of the mechanisms are evolutionary conserved.
Based on the literature research, eight AOPs were identified, describing three connections between the HPG- and HPT-axes (Figure 1), four connections between the HPG- and HPA/I-axes (Figure 2), and one connection between the HPT- and HPA/I-axes (Figure 3).
Even though the developed AOPs represent just a basic structure and data gaps have to be closed at this point, this approach holds high chance for later application to evaluate the endocrine properties of substances.

2. Identification of common KEs and KERs

2.1 AOPs and AOP networks crossing the HPA/I- and the HPG axis

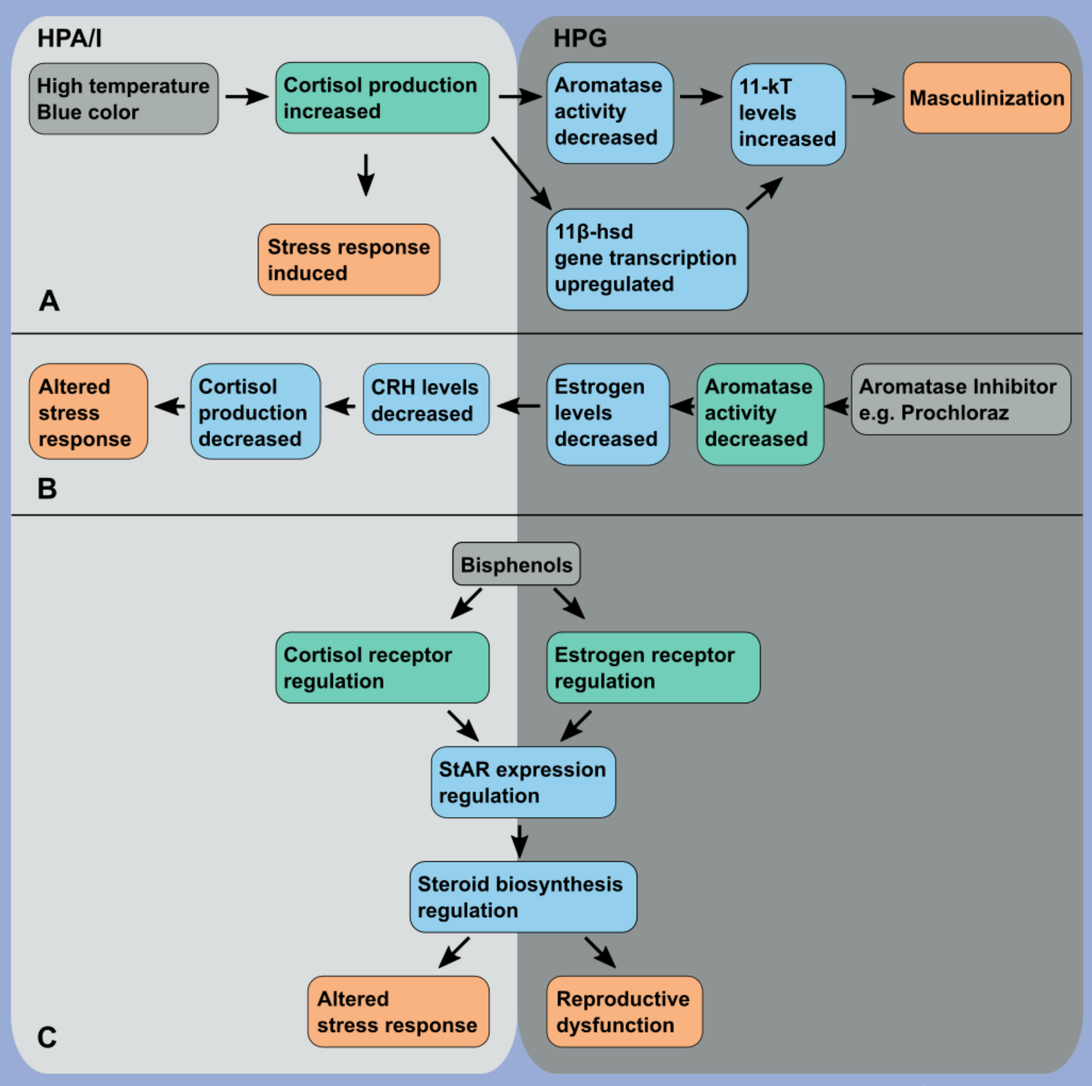

Figure 1: (A) AOP "Increased cortisol levels lead to masculinization in fish". Common KEs are e.g. aromatase inhibition and upregulation of *11β-hsd* gene transcription.
(B) AOP "Aromatase inhibition leads to an altered stress response". The KER bridging between the axes is from decreased estrogen levels resulting in decreased corticotropin releasing hormone (CRH) levels.
(C) AOP "Bisphenol treatment results in altered stress response as well as reproductive dysfunction". Common to both axes is the regulation of steroidogenic acute regulatory protein (StAR) expression.

2.2 AOPs and AOP networks crossing the HPT- and the HPG axis

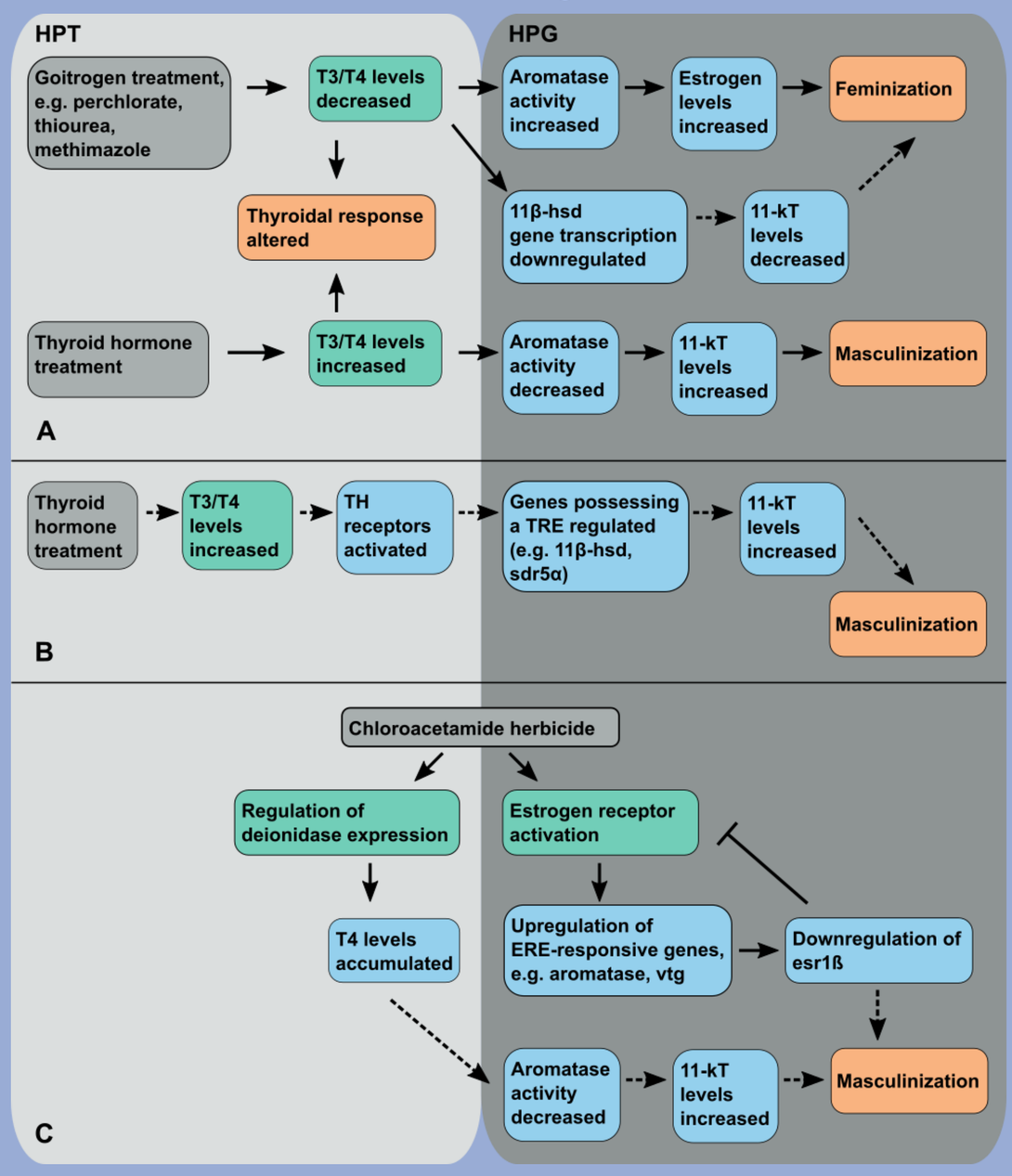

Figure 2: (A) AOP "Decreased thyroid hormone levels lead to feminization"; and accordingly "Increased thyroid hormone levels lead to masculinization". Common KEs are an altered aromatase activity, or reduced *11β-hsd* gene transcription.
(B) AOP "Thyroid hormone receptor activation of genes possessing a TRE results in masculinization". The crosstalk between the axes is mediated via HPG-related genes possessing a thyroid hormone receptor responsive element (TRE) in their promoter region.
(C) AOP "Chloroacetamide herbicide treatment exerts estrogenic as well as thyroidal effects and results in altered endocrine responses". The connection is directly via the substance, which exerts a MIE on both axes. Furthermore, thyroid hormone levels alter the activity of the aromatase enzyme.

2.3 AOP crossing the HPA/I and the HPT- axis, with effects at the HPG axis

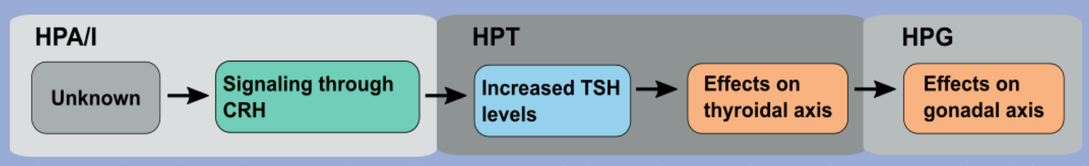

Figure 3: AOP "CRH-mediated increase of thyroid-stimulating hormone (TSH) levels lead to effects on the thyroidal and the gonadal axis". CRH activity results in increased TSH levels, exhibiting indirect effects on the HPG axis.

3. Conclusion

It was possible to define potential AOPs crosslinking the HPG-/HPA/I-axis, the HPG-/HPT-axis, and also the HPA/I-/HPT-axis. In order to apply these AOPs to any testing strategy, some points have to be considered. First, there are several KERs for which no empirical data exist. Second, to be sure that a substance reacts via the described crosslink, at least two KEs, ascribed to both of the axes of interest, have to be assessed. However, even though application of the AOPs could not be performed at this point, there is high potential in application when the data gaps are closed.

Acknowledgements

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In order to provide mechanistic understanding of these interactions, the concept of Adverse Outcome Pathway (AOP) might be used. Therefore, in this study common key events (KE) and key event relationships (KER) shared by two or more endocrine axes were identified by focusing on fish and amphibians, however, data also on mammals were considered, as most of the mechanisms of the three endocrine axes are evolutionary conserved.

This literature review comprises data of ecotoxicological and toxicological studies assessing adverse apical effects, as well as of basic research on physiological processes applying molecular biological approaches. The gathered information delivers data on the interaction of individual biological elements, for example hormone/ hormone receptor interactions, gene transcription regulation, or enzymatic activity. The so identified KEs and KERs provide explanations for unexpected effects on one axis, exerted by substances suspected to act specifically on another axis.

Based on the data of the literature research, eight AOPs were identified. These AOPs describe three connections between the HPG- and HPT-axes, four connections between the HPG- and HPA/I-axes, and one connection between the HPT- and HPA/I-axes. Central KEs identified across axes were altered aromatase activity, and altered expression and function of proteins 11β-Hydroxysteroid Dehydrogenase (11ß-HSD) and Steroidogenic Acute Regulatory (StAR) protein. Substance classes that act on more than one endocrine axis were for example goitrogens like the perchlorates, aromatase inhibitors like prochloraz, or chloroacetamide herbicides.

Even though the developed AOPs represent just a basic structure and data gaps have to be closed at this point, this approach holds high chance for later application to evaluate the endocrine properties of substances.